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Paul D. Taylor
09/756,070
January 16, 2001

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claim 1 (currently amended): A chromatographic method for separating heteroduplex and homoduplex DNA molecules in a mixture, said method comprising:

- (a) applying the mixture to an anion-exchange solid;
- (b) eluting the solid of step (a) with a mobile phase comprising an eluting salt, an organic solvent, and a buffer, wherein said eluting is carried out under conditions effective to at least partially denature said heteroduplexes and wherein the eluting results in the separation of said ~~heteroduplexes~~ heteroduplexes from said homoduplexes.

Claim 2 (original): A method of claim 1 wherein step (b) includes contacting the solid of step (a) with a mobile phase possessing a pH in the range of 4 to 9 said mobile phase comprising:

- an eluting salt composed of equal concentrations of:
 - a cation selected from the group consisting of dialkylammonium, trialkylammonium and tetraalkylammonium, or

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mixtures thereof, wherein the alkyl groups consist of any combination of methyl and ethyl; and

an anion selected from the group consisting of bromide, chloride, acetate, formate, nitrate, perchlorate, dihydrogen phosphate, ethane sulfonate and methane sulfonate or mixtures thereof;

a buffer acid with a pKa in the approximate range of 3.5 to 9.5, and,

an organic solvent;

wherein the concentration of eluting salt is systematically increased from approximately 0.5M to approximately 2.0M,

Claim 3 (original): A method of claim 2 wherein the eluting salt is systematically increased from approximately 1.0M to approximately 2.0M.

Claim 4 (original): A method of claim 2 wherein said cation is selected from the group consisting of dialkylammonium, trialkylammonium and tetraalkylammonium, wherein the alkyl groups consist of any combination of methyl and ethyl.

Claim 5 (currently amended): A ~~method of claim 2~~ chromatographic method for separating heteroduplex and homoduplex DNA molecules in a mixture, said method comprising:

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(a) applying the mixture to an anion-exchange solid;

(b) eluting the solid of step (a) with a mobile phase comprising an eluting salt, an organic solvent, and a buffer, and contacting the solid of step (a) with a mobile phase possessing a pH in the range of 4 to 9 said mobile phase comprising an eluting salt composed of equal concentrations of a cation selected from the group consisting of dialkylammonium, trialkylammonium and tetraalkylammonium, or mixtures thereof, wherein the alkyl groups consist of any combination of methyl and ethyl and an anion selected from the group consisting of bromide, chloride, acetate, formate, nitrate, perchlorate, dihydrogen phosphate, ethane sulfonate and methane sulfonate or mixtures thereof;

(c) a buffer acid with a pKa in the approximate range of 3.5 to 9.5; and

(d) an organic solvent;

wherein the concentration of eluting salt is systematically increased from approximately 0.5 M to approximately 2.0 M, and wherein said eluting is carried out under conditions effective to at least partially denature said heteroduplexes and wherein the eluting results in the separation of said heteroduplexes from said homoduplexes and wherein said cation comprises choline.

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Claim 6 (original): A method of claim 2 wherein said cation comprises guanidinium.

Claim 7 (original): A method of claim 2 where said cation comprises sodium.

Claim 8 (original): A method of claim 2 wherein said anion is formate or chloride.

Claim 9 (original): A method of claim 2 wherein said mobile phase includes a metal chelating agent.

Claim 10 (currently amended): A method of claim 9 wherein said metal chelating agent is selected from the group consisting of acetylacetone, alizarin, aluminon, chloranilic acid, kojic acid, morin, rhodizonic acid, thionalide, thiourea, ~~α -furildioxime~~, nioxime, salicylaldoxime, dimethylglyoxime, α -furildioxime, cupferron, α -nitroso- β -naphthol, nitroso-R-salt, diphenylthiocarbazone, diphenylcarbazone, eriochrome black T, PAN, SPADNS, glyoxal-bis(2-hydroxyanil), murexide, α -benzoinoxime, mandelic acid, anthranilic acid, ethylenediamine, glycine, triaminotriethylamine, thionalide, triethylenetetramine, EDTA, metalphthalein, arsonic acids, α, α' -bipyridine, 4-hydroxybenzothiazole, β -hydroxyquinaldine, β -hydroxyquinoline, 1,10-phenanthroline, picolinic acid, quinaldic acid, α , α' , α'' -

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terpyridyl, 9-methyl-2,3,7-trihydroxy-6-fluorone, pyrocatechol, rhodizonic acid, salicylaldoxime, salicylic acid, tiron, 4-chloro-1,2-dimercaptobenzene, dithiol, mercaptobenzothiazole, rubeanic acid, oxalic acid, sodium diethyldithiocarbarbamate, zinc, dibenzylidithiocarbamate, deferoxamine mesylate, crown ethers, and mixtures of any one or more of the above.

Claim 11 (original): A method of claim 1, wherein said solid is comprised of a silica, polysaccharide or synthetic polyolefin backbone.

Claim 12 (original): A method of claim 11 wherein said polyolefin is a polystyrene or polyacrylic.

Claim 13 (original): A method of claim 1, wherein said solid comprises a polyacrylic backbone.

Claim 14 (original): A method of claim 1, wherein said solid comprises diethylaminoethyl functional groups.

Claim 15 (original): A method of claim 1, wherein said solid comprises polyethyleneimine functional groups.

Claim 16 (original): A method of claim 1, wherein said solid comprises particles with an average diameter between approximately 2 micron and 10 micron.

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Claim 17 (original): A method of claim 1, wherein the solid is substantially nonporous.

Claim 18 (original): A method of claim 1, wherein said solid comprises a polystyrene backbone.

Claim 19 (original): A method of claim 1, wherein said mobile phase contains an organic solvent selected from the group consisting of methanol, ethanol, acetonitrile, ethyl acetate, formamide, 2-propanol, and N-methyl pyrrolidone.

Claim 20 (original): A method of claim 1 wherein said mobile phase contains less than about 40% by volume of said organic solvent.

Claim 21 (original): A method of claim 1 wherein said eluting is carried out at a column temperature greater than about 50°C.

Claim 22 (original): A method of claim 1 wherein said eluting is carried out at a column temperature between about 40°C and about 80°C.

Claim 23 (original): A method of claim 1 wherein the concentration of said eluting salt is continuously increased.

Claim 24 (original): A method of claim 1 including analyzing the mobile phase after the elution step (b) for the concentration of said DNA molecules.

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Claim 25 (original): A method of claim 24 wherein the concentration of said DNA molecules is measured by ultraviolet absorbance in the approximate wavelength range of about 250nm to about 290nm.

Claim 26 (original): A method of claim 1 wherein the total time required to complete said method is between about 2 minutes and about 30 minutes.

Claim 27 (original): A method of claim 1 wherein the concentration of organic solvent is systematically increased.

Claim 28 (currently amended): A method of claim 1 where said solid is contained in a column of cylindrical ~~geometry~~ geometry.

Claim 29 (currently amended): A chromatographic method for separating heteroduplex and homoduplex DNA molecules in a mixture, comprising:

- (a) applying the mixture to an anion-exchange solid,
- (b) eluting the solid of step (a) with a mobile phase containing an eluting salt and a buffer, where said eluting is carried out under conditions effective to at least partially denature said heteroduplexes and where the eluting results in the separation of said ~~heteroduplexes~~ heteroduplexes from said homoduplexes.

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Claim 30 (currently amended): A method of claim 29 wherein step (b) includes contacting the solid of step (a) with a mobile phase possessing a pH in the range of 4 to 9 comprising:

an eluting salt composed of equal concentrations of:

a cation selected from the group consisting of dialkylammonium, trialkylammonium and tetraalkylammonium, wherein the alkyl groups consist of any combination of methyl and ethyl; and

an anion selected from the group consisting of bromide, chloride, acetate, formate, nitrate, perchlorate, dihydrogen phosphate, ethane sulfonate and methane sulfonate; and

a buffer acid with a pKa in the approximate range of 3.5 to 9.5 ;

wherein the concentration of eluting salt is systematically increased from approximately 0.5M to approximately 2.0M.

Claims 31-32 (canceled).

Claim 33 (currently amended): A ~~method of claim 30~~
chromatographic method for separating heteroduplex and homoduplex
DNA molecules in a mixture comprising :

(a) applying the mixture to an anion-exchange solid,

(b) eluting the solid of step (a) with a mobile phase

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containing an eluting salt and a buffer, where said eluting is carried out under conditions effective to at least partially denature said heteroduplexes and where the eluting results in the separation of said heteroduplexes from said homoduplexes and further solid of step(a) with a mobile phase possessing a pH in the range of 4 to 9 comprising:

an eluting salt composed of equal concentrations of a cation selected from the group consisting of dialkylammonium, trialkylammonium, and tetraalkylammonium wherein the alkyl groups consist of any combination of methyl and ethyl;

an anion selected from the group consisting of bromide, chloride, acetate, formate, nitrate, perchlorate, dihydrogen phosphate, ethane sulfonate, and methane sulfonate; and

a buffer acid with a pKa in the approximate range of 3.5 to 9.5; wherein the concentration of eluting salt is systematically increased from approximately 0.5 M to approximately 2.0 M and wherein said cation comprises choline.

Claim 34 (original): A method of claim 30 wherein said cation comprises sodium

Claim 35 (original): A method of claim 30 wherein said mobile phase includes a metal chelating agent.

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Claim 36 (currently amended): A method of claim 35 wherein said metal chelating agent is selected from the group consisting of acetylacetone, alizarin, aluminon, chloranilic acid, kojic acid, morin, rhodizonic acid, thionalide, thiourea, ~~α -furildioxime~~, nioxime, salicylaldoxime, dimethylglyoxime, α -furildioxime, cupferron, α -nitroso- β -naphthol, nitros-R-salt, diphenylthiocarbazone, diphenylcarbazone, eriochrome black T, PAN, SPADNS, glyoxal-bis(2-hydroxyanil), murexide, α -benzoinoxime, mandelic acid, anthranilic acid, ethylenediamine, glycine, triaminotriethylamine, thionalide, triethylenetetramine, EDTA, metalphthalein, arsonic acids, α, α' -bipyridine, 4-hydroxybenzothiazole, β -hydroxyquinaldine, β -hydroxyquinoline, 1,10-phenanthroline, picolinic acid, quinaldic acid, α , α' , α'' -terpyridyl, 9-methyl-2,3,7-trihydroxy-6-fluorone, pyrocatechol, rhodizonic acid, salicylaldoxime, salicylic acid, tiron, 4-chloro-1,2-dimercaptobenzene, dithiol, mercaptobenzothiazole, rubeanic acid, oxalic acid, sodium ~~diethyldithiocarbamate~~ diethyldithiocarbamate, zinc dibenzylidithiocarbamate, deferoxamine mesylate, crown ethers, and mixtures of any one or more of the above.

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Claim 37 (original): A method of claim 30 wherein said cation comprises guanidinium.

Claim 38 (original): A method of claim 30 wherein said anion is formate or chloride.

Claim 39 (original): A method of claim 30 wherein the eluting salt is systematically increased from approximately 1.0M to approximately 2.0M.

Claim 40 (original): A method of claim 30 including analyzing the mobile phase eluting from the column for the presence of DNA.

Claim 41 (original): A method of claim 30 wherein said eluting is carried out at a column temperature greater than about 50°C.

Claim 42 (original): A method of claim 30 wherein said eluting is carried out at a column temperature between about 40°C and about 80°C.

Claims 43-63 (canceled).

Claim 64 (currently amended): A chromatographic method for separating heteroduplex and homoduplex DNA molecules in a mixture, said method comprising:

- (a) applying the mixture to an anion-exchange solid;
- (b) eluting the solid of step (a) with a mobile phase containing an eluting salt, an organic solvent, and a buffer,

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wherein said eluting is carried out under conditions effective to at least partially denature said heteroduplexes and wherein the eluting results in the separation of said ~~heteroduplexes~~ heteroduplexes from said homoduplexes;

wherein step (b) includes contacting the solid of step (a) with a mobile phase possessing a pH in the range of 4 to 9 comprising:

an eluting salt comprising of equal concentrations of:

a cation;

an anion;

a buffer acid with a pKa in the approximate range of 3.5 to 9.5; and

an organic solvent;

wherein said mobile phase contains less than about 40% by volume of said organic solvent;

wherein the concentration of eluting salt is systematically increased from approximately 0.5M to approximately 2.0M.

Claim 65 (canceled).

Claim 66 (original): A chromatographic method for separating heteroduplex and homoduplex DNA molecules in a mixture, comprising:

(a) applying the mixture to an anion-exchange solid;

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(b) eluting the solid of step (a) with a mobile phase comprising an eluting salt, an organic solvent, and a buffer, wherein said eluting is carried out under conditions effective to at least partially denature said heteroduplexes and wherein the eluting results in the separation of said heteroduplexes from said homoduplexes;

wherein step (b) includes contacting the solid of step (a) with a mobile phase possessing a pH in the range of 4 to 9 comprising an eluting salt comprising:

betaine at a concentration in the range of about 0.5M to about 6M;

a buffer acid with a pKa in the approximate range of 3.5 to 9.5; and,

an organic solvent;

wherein said mobile phase contains less than about 40% by volume of said organic solvent;

wherein the concentration of eluting salt is systematically increased from approximately 0.5M to approximately 2.0M.

Claim 67 (original): A method of claim 66 wherein the eluting is carried out at a column temperature greater than about 50°C.

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Claim 68 (currently amended): A chromatographic method for separating heteroduplex and homoduplex DNA molecules in a mixture, said method comprising:

(a) applying the mixture to an anion-exchange solid;

(b) eluting the solid of step (a) with a mobile phase containing an eluting salt, an organic solvent, and a buffer, where said eluting is carried out under conditions effective to at least partially denature said heteroduplexes and where the eluting results in the separation of said ~~heteroduplexes~~ heteroduplexes from said homoduplexes;

wherein step (b) includes contacting the solid of step (a) with a mobile phase possessing a pH in the range of 4 to 9 comprising:

an eluting salt comprising equal concentrations of :

a cation;

an anion;

a buffer acid with a pKa in the approximate range of 3.5 to 9.5; and wherein the eluting is carried out at a column temperature greater than about 50°C,

wherein the concentration of eluting salt is systematically increased from approximately 0.5M to approximately 2.0M.

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Claim 69-71 (canceled).

Claim 72 (original): The method of claim 1, where prior to said applying step the DNA molecules are amplified using the polymerase chain reaction and the amplified DNA molecules are denatured and renatured to form a mixture of heteroduplex and homoduplex DNA molecules.

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REMARKS

The Examiner has indicated that claims 1-72 are pending. However, claims 43-63 and 69-71 were canceled in the response to restriction requirement filed December 13, 2002. Therefore, claims 1-42, 64-68 and 72 are pending in the instant application. The subject matter of claims 5 and 33 has been acknowledged by the Examiner to be allowable. Claims 31, 32 and 65 have been canceled. Claims 1, 5, 10, 28, 29, 30, 33, 36, 64, and 68 have been amended. No new matter has been added by these amendments. Reconsideration is respectfully requested in light of these amendments and the following remarks.

I. Rejection of Claim 65 under 35 U.S.C. § 112, second paragraph

Claim 65 has been rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 65 is dependent on non-elected claim 63 and the metes and bounds of claim 65 are suggested to be unclear.

Applicant respectfully disagrees that the metes and bounds of this claim are unclear, however, in an attempt to facilitate prosecution, claim 65 has been canceled. Withdrawal of this

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rejection under 35 U.S.C. § 112, second paragraph is respectfully requested in light of the amendments to the claim and the above remarks.

II A-D. Rejection of Claims under 35 U.S.C. § 103(a)

A. Claims 1-4, 7-13, 17-36, 38-42, 64, 66, 67 and 68

Claims 1-4, 7-13, 17-36, 38-42, 64, 66, 67 and 68 have been rejected under 35 U.S.C. § 103(a) over Ohmiya et al. (Analytical Biochemistry (1990) Vol. 189, pages 126-130) in view of Gjerde et al. (U.S. Patent 5,972,222).

Ohmiya et al. is suggested to teach the separation of various types of DNA molecules and their fragments of various sizes by (a) applying DNA molecules to an anion exchange solid. The Examiner acknowledges that Ohmiya does not teach a chromatographic method for separating heteroduplex and homoduplex DNA molecules in a mixture. The Examiner further acknowledges that Ohmiya et al. do not teach a chromatographic method, wherein the eluting salt comprises a cation selected from di- or trialkylammonium and a buffer acid with a pKa in the approximate range of 3.5 to 9.5 and an organic solvent. The Examiner yet further acknowledges that Ohmiya et al. does not teach a chromatographic method wherein the

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mobile phase contains an organic solvent acetonitrile which is less than about forty percent by volume of the organic solvent, nor does it teach an average solid diameter between approximately 2 microns and 10 microns. The Examiner yet further acknowledges that Ohmiya et al. do not teach a chromatographic method wherein the concentration of the organic solvent is systematically increased.

It is further acknowledged that Ohmiya et al. does not teach a chromatographic method wherein the eluting is carried out at a column temperature greater than about 50 degrees centigrade and in between 40 to 80 degree centigrade.

The Examiner suggests that Gjerde et al. teach a chromatographic method for separating heteroduplex and homoduplex DNA molecules in a mixture (Examples 7, and Figures 14 and 15) the method comprising eluting the solid of ion exchange column with a mobile phase comprising an eluting salt an organic solvent and a buffer, wherein the eluting is carried out under conditions effective to at least partially denature the heteroduplexes and wherein the eluting results in the separation of the heteroduplexes from the homoduplexes.

Gjerde et al. is suggested to teach a chromatographic method wherein the concentration of the organic solvent is systematically

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increased and wherein the eluting is carried out at a column temperature greater than about 50 degrees centigrade and in between 40 to 80 degrees centigrade.

The Examiner suggests that it would have been *prima facie* obvious to one having ordinary skill in the art at the time the invention was made to combine and substitute the method in which homoduplex and heteroduplex double stranded polynucleotides are separated by the method of Gjerde in the high resolution anion exchange chromatographic method of Ohmiya et al., since Gjerde states "the methods used to capture multivalent cations and prevent their presence in the batch process. . . are essential in order to achieve high resolution separations of polynucleotides, especially double stranded DNA and also to greatly extend the useful life of the separation media". It is suggested that an ordinary practitioner would have been motivated to combine and substitute the method in which homoduplex and heteroduplex double stranded polynucleotides are separated as taught by Gjerde et al., in the high-resolution anion exchange chromatographic method of Ohmiya et al. in order to achieve the express advantages of an invention which provides a method to achieve high resolution separations of

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polynucleotides especially double stranded DNA, and also to achieve the express advantages of the nonporous QA column.

The Examiner noted that neither Ohmiya et al., nor Gjerde et al., teach a method wherein the total time required to complete the method is between about 2 minutes and about 30 minutes. The Examiner suggests that it is *prima facie* obvious that selection of the specific time to finish a polynucleotide purification procedure represents routine optimization with regard to the amount of DNA molecules present in the sample to be purified and the flow rate of the columns.

Applicant respectfully disagrees with this rejection.

Rights in the present invention are assigned to Transgenomic, Inc. of San Jose California. Similarly, rights to Gjerde et al. (U.S. Patent 5,972,222) were assigned to Transgenomic, Inc. of San Jose, CA. Under MPEP section 301 (regarding the ownership and assignability of patents and applications) and further under 35 U.S.C. 261 patents shall have the attributes of personal property. Applications for patent, patents, or any interest therein, shall be assignable in law by an instrument in writing. The applicant, patentee, or his assigns or legal representatives may in like manner grant and convey an exclusive right under his application

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for patent, or patents, to the whole or any specified part of the United States

Under 35 U.S.C. 103 (c), "[s]ubject matter developed by another person, which qualifies as prior art only under one or more of subsections (e), (f), and (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person". Accordingly the Gjerde patent, assigned to Transgenomic Inc., is not a proper prior art reference and cannot be properly combined with the Ohmiya reference under 35 USC 103. The Examiner has acknowledged that Ohmiya alone does not teach or suggest the present claimed invention. Accordingly, withdrawal of this rejection is respectfully requested.

B. Claims 6, 37, and 72

Claims 6, 37, and 72 are rejected under 35 U.S.C. 103(a) as being obvious over Ohmiya (Analytical Biochemistry, (1990) Vol. 189, pages 126-130) in view of Gjerde (U.S. Patent 5,972,222) and further in view of Bertling (U.S. Patent 6,306,592).

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Ohmiya, in view of Gjerde et al., are suggested to teach a method wherein the cation comprises guanidinium. Bertling is further suggested to teach a method wherein the cation comprises guanidinium. It is acknowledged that Ohmiya, in view of Gjerde et al., do not teach a method wherein prior to the applying step the DNA molecules are amplified using the polymerase chain reaction and the amplified DNA molecules are denatured and renatured to form a mixture of heteroduplex DNA molecules.

Bertling is suggested to teach a method wherein prior to the applying step the DNA molecules are amplified using the polymerase chain reaction and the amplified DNA molecules are denatured and renatured to form a mixture of heteroduplex and homoduplex DNA molecules. Bertling is further suggested to teaches a method wherein prior to the applying step the DNA molecules are amplified using the polymerase chain reaction and the amplified DNA molecules are denatured and renatured to form a mixture of heteroduplex and homoduplex DNA molecules.

The Examiner suggests that it would have been *prima facie* obvious to one having ordinary skill when the invention was made to combine and substitute the method, wherein the cation comprises guandidinium and wherein prior to the applying step the DNA

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molecules are amplified using the polymerase chain reaction and the amplified DNA molecules are denatured and renatured to form a polymerase chain reaction and the amplified DNA molecules are denatured and renatured to form a mixture of heteroduplex and homoduplex DNA molecules of Bertling in the high-resolution anion exchange chromatographic method of Ohmiya, in view of Gjerde et al., since Bertling states "the method is especially sensitive when the purification step comprises a chromatographic purification method. The chromatographic purification method may be a column or a batch method which is carried out using matrices such as silica gel or DEAE material, all of which allow a separation on the principle of ion exchange, affinity size exclusion".

Applicant respectfully traverses this rejection.

As set forth above, Gjerde et al. is not a valid prior art reference. Further, Bertling is not a proper prior art reference because the priority date of the present invention is April 4, 2000. Thus, neither Gjerde nor Bertling may be combined with Ohmiya under 35 U.S.C. 103(a). The Examiner has acknowledged, *supra*, that Ohmiya alone does not disclose claims 6, 37, and 72 of the claimed invention. Thus the invention cannot be deemed obvious.

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Withdrawal of this rejection is respectfully requested.

C. Claims 14 and 15

Claim 14 and 15 are rejected under 35 USC 103(a) as being obvious over Ohmiya, in view of Gjerde et al., and further in view of Cohen et al. (U.S. Patent 5,506,103).

Ohmiya, in view of Gjerde is suggested to teach a method wherein the solid comprises diethylaminoethyl and polyethyleneimine functional groups.

Cohen et al., is suggested to supply or teach a method wherein the solid comprises diethylaminoethyl and polyethyleneimine functional groups.

It is suggested that it would have been *prima facie* obvious to one having ordinary skill in the art at the time the invention was made to combine and substitute the method, wherein the solid comprises diethylaminoethyl and polyethyleneimine functional groups of Cohen et al., in the high-resolution anion exchange chromatographic method of Ohmiya, in view of Gjerde, since Cohen et al. states that "another useful resin is a weak anion exchange resin such as diethylaminoethyl and polyethyleneimine. An ordinary practitioner is suggested to have been motivated to combine and

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substitute the diethylaminoethyl and polyethyleneimine functional groups of Cohen et al., in the high resolution anion exchange chromatographic method of Ohmiya et al., in view of Gjerde, to achieve an invention which provides a useful anion exchange resin such as diethylaminoethyl and polyethyleneimine.

Applicant respectfully disagrees with this rejection under 35 U.S.C. §103(a).

As set forth above, the Gjerde reference cannot be used as a prior art reference under 35 U.S.C. §103(a). Further, the Examiner has acknowledged that neither Ohmiya, nor Cohen alone or combined, can be construed to teach or suggest the present invention. As neither Ohmiya nor Cohen teach or suggest the chromatographic method of claim 1 of the instant invention, for separation of heteroduplex and homoduplex DNA molecules in a mixture, these references cannot be deemed to make obvious dependent claims 14 and 15 of the present invention.

Withdrawal of this rejection is respectfully requested.

D. Claim 16

Claim 16 is rejected under 35 U.S.C. 103(a) as being obvious over Ohmiya et al., in view of Gjerde et al., and further in view

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of Ausserer et al. (Biotechniques (1995) Vol. 19, No.1, pages 136-139). Ohmiya et al., in view of Gjerde is suggested to teach the method of claims 1-4, 7-13, 17-36, 38-42, 64, 66, 67, and 68, of the present invention. The Examiner acknowledges that Ohmiya et al., in view of Gjerde et al., do not teach a method wherein the solid comprises particles with an average diameter between approximately 2 microns and 10 microns. Ausserer is used to suggest a method wherein the solid comprises particles with an average diameter between approximately 2 micron and 10 micron. The Examiner suggests that it would have been *prima facie* obvious to one of skill at the time the invention was made to combine and substitute the method wherein the solid comprises particles with an average diameter between approximately 2 micron and 10 micron of Ausserer et al.

Applicant respectfully disagrees.

As recited in detail *supra*, the Gjerde reference cannot be used as a valid prior art reference under 35 U.S.C. §103(a), as it is not considered to be the work of another. Further, the Examiner has acknowledged that Ohmiya alone does not disclose the claimed invention. As Ausserer is recited simply for its use of a particle size between approximately 2 micron and 10 microns, Ausserer does

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not supply Ohmiya with any other requisite teachings to teach or suggest the present invention. Accordingly the recited combination can not be deemed to make obvious claim 16 of the present invention.

Withdrawal of this rejection is respectfully requested.

III. Allowable Subject Matter

Claims 5 and 33 are objected to as being dependent upon a rejected base claim, but were acknowledged to be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims.

Applicant has amended claims 5 and 33 as suggested by the Examiner to independent formats including all of the limitations of the base claim and any intervening claims. Support for this amendment is found throughout the specification, and by originally filed claims 1, 2, 29, and 30. Allowance of these claims is respectfully requested.

IV. Typographical errors

Claims 1, 10, 28, 29, 30, 36, 64 and 68 have been amended by Applicant to correct typographical errors in spelling. Support for

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these amendments is found throughout the specification. No new matter has been added.

Further, in an earnest attempt to facilitate and streamline prosecution, Claims 31 and 32 have been canceled.

V. Conclusion

Applicant believes that the foregoing comprises a full and complete response to the Office Action of record. Accordingly, favorable reconsideration and subsequent allowance of the pending claims is earnestly solicited.

Respectfully submitted,


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